FORM-PTO-1390 (Rev. 12-29-99) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 003300-782

U.S APPLICATION NO (If known, see 37 C F R 1.5): 09/856576

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/SE99/02197	25 November 1999	25 November 1998

TITLE OF INVENTION
MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS

APPLICANT(S) FOR DO/EO/US
PETER ERIKSSON

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

The Preliminary Amendment filed herewith cancels Claims 1 to 31 that were submitted on 12 February 2001 during the international phase of examination and adds new Claims 32 to 48.

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Signed Declaration will follow).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information: A certified copy of Swedish Application No. 9804064-5, filed 25 November 1998, was submitted during the international phase of examination. Thus the claim for priority has been ferfected.

A Transmittal of Formal Drawings is filed herewith.

JC18 Rec'd PCT/PTO 23 MAY 2001

APPLICATION NO. (If known, see 37 CFR 1.5(a)) 09/856576	INTERNATIONAL APPLICATION NO. PCT/SE99/02197	ATTORNEY'S DOCKET NUMBER 003300-782

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1,000.00 (960)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $860.00 (970)		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $710.00 (958)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $690.00 (956)		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00 (962)		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 1,000.00	
Surcharge of **$130.00 (154)** for furnishing the oath or declaration later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(e)).	$ ----	

Claims	Number Filed	Number Extra	Rate		
Total Claims	27 -20 =	7	X$18.00 (966)	$ 126.00	
Independent Claims	5 -3 =	2	X$80.00 (964)	$ 160.00	
Multiple dependent claim(s) (if applicable)			+$270.00 (968)	$ ----	
			TOTAL OF ABOVE CALCULATIONS =	$ 1,286.00	
Reduction for 1/2 for filing by small entity, if applicable (see below).				$ 643.00	-
			SUBTOTAL =	$ 643.00	
Processing fee of **$130.00 (156)** for furnishing the English translation later than 20 ☐ 30 ☐ months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ ----	
			TOTAL NATIONAL FEE =	$ 643.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 (581)** per property +				$ ----	
			TOTAL FEES ENCLOSED =	$ 643.00	
			Amount to be: refunded	$	
			charged	$	

a. ☒ Small entity status is hereby claimed.

b. ☒ A check in the amount of $ 643.00 to cover the above fees is enclosed.

c. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

d. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Benton S. Duffett, Jr.
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Filed: May 23, 2001

Benton S. Duffett, Jr.
SIGNATURE

Benton S. Duffett, Jr.
NAME

22,030
REGISTRATION NUMBER

Patent
Attorney's Docket No. 003300-782

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	) Box PCT
PETER ERIKSSON	) Attention: DO/EO/US
Application No.: Unassigned (Corresponds to PCT/SE99/02197)	) Group Art Unit: Unassigned
Filed: May 23, 2001	) Examiner: Unassigned
For: MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS	)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, please amend the above-captioned application as follows:

IN THE ABSTRACT:

Please add the Abstract of the Disclosure that is provided on a separate sheet.

IN THE CLAIMS:

Kindly cancel claims 1 to 34 as published and Claims 1 to 31 that were submitted on February 12, 2001 during the International phase of examination, and insert the following in lieu thereof new claims 32 to 58.

32. (New) A method for the treatment of CNS damage affecting neural stem cells, progenitor cells and/or cells derived from stem cells or progenitor cells comprising

administering an effective amount of growth hormone or a functionally equivalent analog thereof to a patient in need of such treatment.

33. (New) The method according to Claim 32, wherein said CNS damage affects the oligodendroglia, astroglia, and/or neuronal cells.

34. (New) The method according to Claim 32, wherein said CNS damage affects non-cholinergic neuronal cells, cholinergic neuronal cells, or glial cells.

35. (New) The method according to Claim 32, wherein said CNS damage is neural cell loss.

36. (New) The method according to Claim 32, wherein said CNS damage is caused by hypoxic injury, ischemic injury, and/or traumatic injury.

37. (New) The method according to Claim 32, wherein said medicinal product is formulated for intravenous infusion, intramuscular injection or subcutaneous injection.

38. (New) The method according to Claim 32, wherein said medicinal product is formulated so that the active substance will pass into the ventricles of the patient's brain when it is administered to a patient.

39. (New) The method according to Claim 32, wherein said medicinal product is formulated so that the active substance will pass into the cerebrospinal fluid of the patient when it is administered to a patient.

40. (New) A method for the treatment of an abnormal condition affecting the central nervous system, wherein said abnormal condition is the consequence of axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, damage to the spinal cord after disease and/or trauma comprising administering an effective amount of a substance to a patient whereupon administration of said substance will lead to a decreased concentration of growth hormone or a functionally equivalent analog thereof.

41. (New) The method according to Claim 40, wherein said substance is a negatively regulating growth hormone binding protein, a functionally equivalent analogous thereof, an antibody against growth hormone, a biologically active growth hormone receptor inhibitor, and/or an inhibitor of endogenous growth hormone release.

42. (New) A method of propagating progenitor cells, stem cells and/or cells derived from said cells by administration of an effective amount of growth hormone or a functionally equivalent analogue thereof to stem cells, progenitor cells, neurons astroglial cells and/or oligodendrocytes in vitro.

43. (New) A method of inducing lineage determination or inducing or maintaining the genesis of neurons, oligodendrocytes, astroglia cells from progenitor cells or stem cells in, or derived from, the central or peripheral nervous system in a patient, wherein a pharmaceutically effective amount of growth hormone or a functionally equivalent analogue thereof is administered to a patient in need thereof.

44. (New) A method according to Claim 43, for treatment of an abnormal condition affecting the nervous system of a patient.

45. (New) A method according to Claim 44, wherein said condition affects the oligodendroglia, astroglia, and/or neuronal cells.

46. (New) A method according to Claim 44, wherein said condition affects the non-cholinergic neuronal cells, cholinergic neuronal cells, or glial cells.

47. (New) A method according to Claim 44, wherein said condition is a CNS damage or deficit.

48. (New) A method according to Claim 47, wherein said condition is neural cell loss.

49. (New) A method according to Claim 47, wherein said condition is memory loss.

50. (New) A method according to Claim 47, wherein said condition is caused by at least one factor selected from the group consisting of multiple sclerosis, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, and demyelinating disorder.

51. (New) A method according to Claim 43, wherein said substance is administered by intravenous infusion, intramuscular injection or subcutaneous injection.

52. (New) A method according to Claim 43, wherein brain cells are removed from the patient after said administration, said brain cells then being propagated in vitro, followed by transplantation of the obtained cells back into the brain of a patient in need thereof.

53. (New) A method according to Claim 52, wherein an effective amount of growth hormone or a functionally equivalent analogue thereof is administered to said brain cells during in vitro propagation.

54. (New) A method of reducing the genesis of oligodendrocytes, neurons, astroglia cells from progenitor cells or stem cells in, or derived from, the central or peripheral nervous system in a patient, wherein a pharmaceutically effective amount of a substance that will lead to a decreased concentration of growth hormone or a functionally equivalent analogue thereof is administered to a patient in need thereof.

55. (New) A method according to Claim 54, wherein said substance is administration to the peripheral or central nervous system of said patient.

56. (New) A method according to Claim 54, wherein said substance is selected from the group consisting of negatively regulating growth hormone binding proteins, functionally equivalent analogous thereof, antibodies against growth hormone, biologically active growth hormone receptor inhibitors, and inhibitors of endogenous GH release.

57. (New) A method according to Claim 54, for treatment of a central nervous system injury.

58. (New) A method according to Claim 57, wherein said injury is the consequence of a factor selected from the group consisting of axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, damage to the spinal cord after disease or trauma.

REMARKS

Entry of the foregoing amendments is respectfully requested. The examination and allowance of the applications are urged to herein order and are respectfully requested.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Benton S. Duffett, Jr.
Registration No. 22,030

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: May 23, 2001

ABSTRACT OF THE DISCLOSURE

Use of a substance that upon administration will lead to increased concentrations of growth hormone, such as growth hormone, a functionally equivalent analogue thereof or a substance that will increase the release of endogenous growth hormone, for the production of a medicinal product for treatment of abnormal conditions affecting neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells, especially conditions affecting the oligodendroglia, astroglia, and/or neuronal cells. In vitro and in vivo methods for inducing lineage determination, propagating and/or inducing or maintaining the genesis of neurons, oligodendrocytes, astroglial cells from progenitor cells, stem cells and/or cells derived from said cells by administrating to the cells a substance that increases the concentration of growth hormone. Also a method of reducing the genesis of oligodendrocytes, neurons, astroglial cells from progenitor cells or stem cells, wherein a pharmaceutically effective amount of a substance that will lead to a decreased concentration of growth hormone or a functionally equivalent analogue thereof is administered to said patient.

Patent
Attorney's Docket No. 003300-782

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	)	Box PCT
	)	
PETER ERIKSSON	)	Attention: DO/EO/US
	)	
Application No.: Unassigned (Corresponds to PCT/SE99/02197)	)	Group Art Unit: Unassigned
	)	
	)	Examiner: Unassigned
Filed: May 23, 2001	)	
	)	
For: MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS	)	

TRANSMITTAL OF FORMAL DRAWINGS

Assistant Commissioner for Patents
Washington, D.C. 20231

ATTN: OFFICIAL DRAFTSMAN

Sir:

Applicant submits herewith two (2) sheets of formal drawings (i.e., Figs. 1 to 3).

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Benton S. Duffett, Jr.
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P.O. Box 1404
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Date: May 23, 2001

1/2

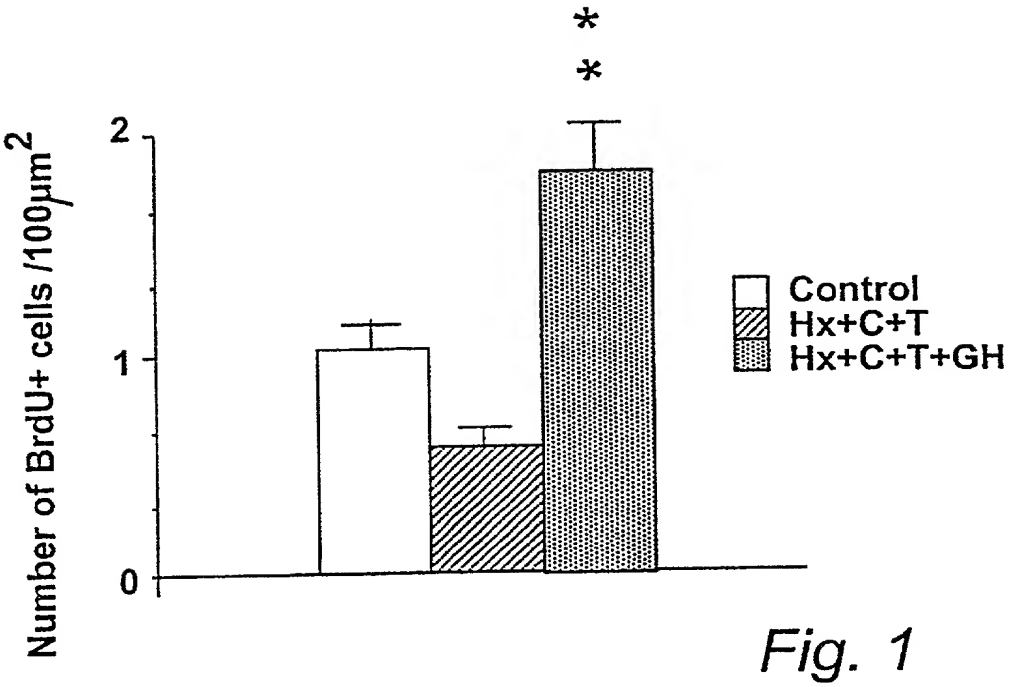
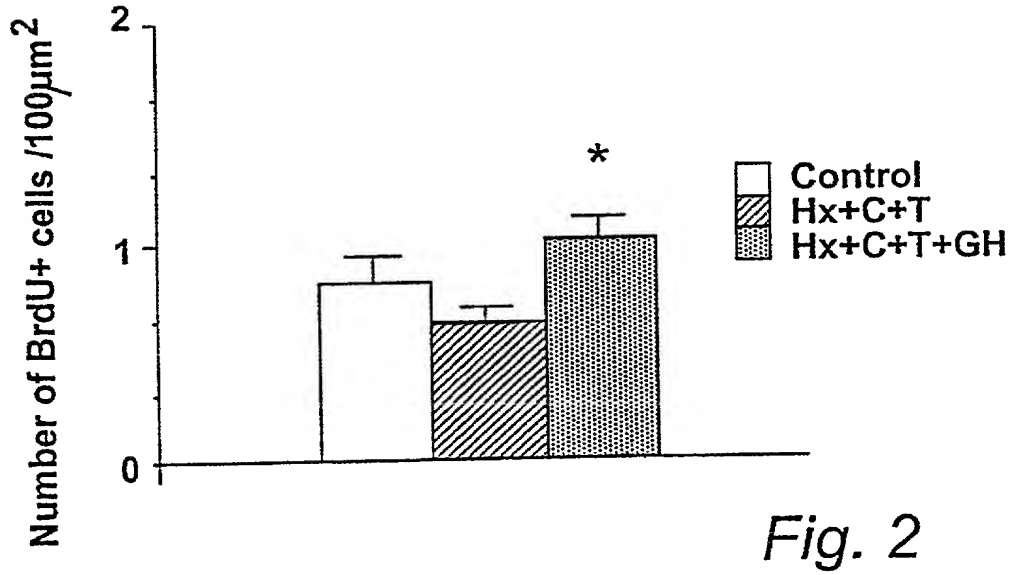

*
*
2
1
0
Number of BrdU+ cells /100µm2
Control
Hx+C+T
Hx+C+T+GH

Fig. 1


2
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Number of BrdU+ cells /100µm2
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Hx+C+T
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Fig. 2

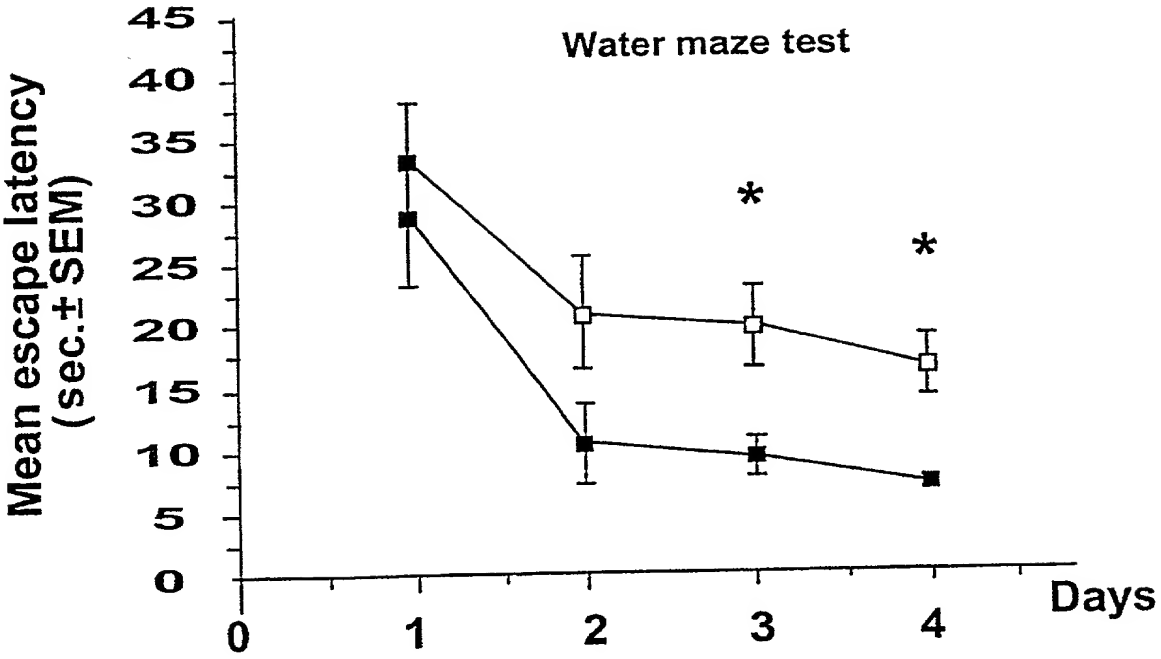
Water maze test
Mean escape latency (sec.± SEM)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
Days
*
*

Fig. 3

MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS

Field of invention

The present invention relates to use of substances that upon administration to a patient will lead to increased concentrations of growth hormone for the production of medicinal products.

The present invention also relates to a method for treatment of abnormal conditions affecting neural stem cells or progenitor cells.

Background of the invention

The result of traumatic, asfyxial, hypoxic, ischemic, toxic, infectious, degenerative or metabolic insults to the central nervous system (CNS) of man may involve a certain degree of damage in several different cell types. Damage to the brain by trauma, asphyxia, toxins, ischemia or infections are frequently causing neurological and cognitive deficits. Degenerative diseases may cause loss of specific populations of cells. For instance Parkinson's disease is associated by specific loss of dopaminergic neurons in the Substantia nigra, similarly, multiple sclerosis is associated with loss of myelin and oligodendrocytes. Other examples of degenerative disorders caused by selective loss of a specialized type of neurons is Alzheimer's disease associated with loss of cholinergic neurons. There are many other instances in which CNS injury or disease can cause damage to oligodendroglia, astroglia or neuronal cells.

Furthermore, axonal regeneration and sprouting after injury to axons in the CNS white mater tracts and injury to the spinal cord has been shown to be inhibited by surface molecules expressed by oligodendrocytes.

Progenitor cells have been grown and propagated with growth factors like epidermal growth factor (EGF), which is a substance belonging to a different class than GH.

In general, replacement of neurons following degeneration or damage is not a characteristic of the mammalian brain. Neuronal loss is thus considered permanent. Prolonged postnatal neurogenesis has been described in the granule cell layer of the hippocampal formation (Altman, J., and Das, G.D. , J. Comp. Neurol. 124: 319-335 (1965); Altman, J. and Das, G.D. Nature 214: 1098-1101 (1967); Caviness, V. S. jr., J. Comp Neurol. 151: 113-120 (1973); Gueneau, G., Privat, A., Drouet, J., and Court, L., Dev. Neurosci. 5, 345-358(1982); Eckenhoff, M.F., and Rakic, P., J. Neurosci. 8: 2729-2747(1988)). Neurogenesis has recently been shown to persist well into adulthood in man (Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A., Nordborg, C., Peterson, D.A., Gage, F.H., Nature Med. in press). Neuronal progenitor cells reside in the subgranular zone (SGZ) of the dentate gyrus where they continuously proliferate, migrate into the granulae cell layer and differentiate into granule cells (Kuhn, H., Dickinson-Anson, H., and Gage, F.H., J. Neurosci. 16: 2027-2033 (1996); Cameron, H.A., Woolley, C.S., McEwen, B.S., and Gould, E., Neuroscience 56: 337-344 (1993); Seki, T. and Arai, Y., J. Neurosci. 13: 2351-2358 (1993)). These newborn neurons in the granule cell layer express markers of differentiated neurons and have morphological characteristics corresponding to differentiated granulae cells (Kaplan, M.S. and Bell, D.H., J. Neurosci. 4: 1429-1441 (1984); Cameron, H.A., Woolley, C.S., McEwen, B.S. and Gould, E. Neuroscience 56: 337-344 (1993); Cameron, H.A., Woolley, C.S., and Gould, E., Brain Res. 611: 342-346 (1993)). Furthermore, they establish axonal processes into the mossy fiber pathway and form synaptic connections with their targets in hippocampus CA3 (Seki, T. and Arai, Y., J. Neurosci. 13: 2351-2358 (1993); Stanfield, B.B. and Trice, J.E. Exp. Brain

Res. 72: 399-406 (1988)). The hippocampus is associated with spatial learning and memory (McNamara, R.K, and Skelton, R.W., Brain Res. Rev. 18: 33-49 (1993)). The proliferation of progenitor cells can be influenced by the administration of n-methyl-d-aspartate (NMDA) receptor antagonists or by the removal of the adrenal glands (Cameron, H.A. and Gould, E. Neuroscience 61: 203-209 (1994); Cameron, H.A., Tanapat, P., and Gould, E., Neuroscience 82: 349-354 (1998)). Plasticity is reduced with increasing age, and recent studies have demonstrated that proliferation of progenitor cells also is decreased but not completely abolished with age (Kuhn, H., Dickinson-Anson, H., and Gage, F.H., J. Neurosci. 16: 2027-2033 (1996)). Stem cells isolated from the adult rodent brain has recently been transplanted into the brain of adult animals where they differentiate into cells with neuronal characteristics (Suhonen, J.O., Peterson, D.A., Ray, J. And Gage, F.H., Nature 383:624-627 (1996)).

Furthermore, neurogenesis in the dentate gyrus in young mice has been shown to be facilitated by enriched environments. It was shown that exposure to enriched environments leads to an increased number of surviving newly formed granulae cell neurons and an increased total number of neurons in the dentate gyrus (Kempermann, G., Kuhn, H. G., and Gage, F. H., Nature 386: 493-495 (1997)).

Summary of the invention

It has now been found that by using growth hormone, or an analogue thereof, or another substance leading to increased concentrations of growth hormone or analogues thereof, it is possible to modulate the proliferation and/or differentiation of neural stem cells and progenitor cells from the adult CNS. The present invention thus provides new possibilities to treat injuries to or diseases of the central nervous system that predominantly affect oligodendroglia, astroglia or neuronal cells by

modification of proliferation cell genesis and/or differentiation of neuronal stem cells or progenitor cells in the central nervous system.

It has also been found that it is possible to control the propagation in vitro of stem cells, progenitor-cells and other cells, especially cells derived from the central nervous system, with the potential to generate neurons, astrocytes or oligodendrocytes. Such cells may e.g. be used for therapeutic purposes in patients.

Thus, the present invention relates to the use of a substance that upon administration to a patient will lead to an increased concentration of growth hormone or a functionally equivalent analogue thereof for the production of a medicinal product for treatment of an abnormal condition affecting neural stem cells and/or progenitor cells.

The invention also relates to a method for treatment of an abnormal condition affecting neural stem cells and/or progenitor cells, wherein a pharmaceutically active amount of a substance that will lead to an increased concentration of growth hormone or a functionally equivalent analogue thereof is administered to a patient.

Furthermore, the invention relates to a method of inducing lineage determination, propagating and/or inducing or maintaining the genesis of neurons, oligodendrocytes, astroglial cells from progenitor cells, stem cells and/or cells derived from said cells by administration of an effective amount of growth hormone or a functionally equivalent analogue thereof to stem cells, progenitor cells, neurons astroglial cells and/or oligodendrocytes in vitro.

Another aspect of the invention relates to abnormal conditions in the CNS due to too high concentrations of growth hormone in the CNS.

The invention thus also relates to the use of a substance that upon administration to a patient will lead to a decreased concentration of growth hormone or a func-

tionally equivalent analogue thereof for the production of a medicinal product for treatment of an abnormal condition affecting stem cells, progenitor cells and/or cells derived from stem cells or progenitor cells, as well as to a method of reducing the genesis of oligodendrocytes, neurons, astroglial cells from progenitor cells or stem cells in, or derived from, the central or perifeeral nervous system in a patient, wherein a pharmaceutically effective amount of a substance that will lead to a decreased concentration of growth hormone or a functionally equivalent analogue thereof is administered to said patient.

The characterizing features of the invention will be evident from the following description and the appended claims.

Detailed description of the invention

The mammalian brain, including the human brain, retains its ability to generate neurons throughout life in certain brain regions. New neurons and astroglial cells and oligodendrocytes are generated by cell genesis from stem or progenitor cells. During the research leading to the present invention it was found that growth hormone (below denoted GH) induces an increase in cell genesis from progenitors/stem cells in the adult brain. It was also found that increased number of new cells in the hippocampus is associated with improvement in learning and memory. These findings lead to the insight that it is possible to manipulate neurological deficits, such as memory and learning deficits, in patients by manipulating the amount of GH present in the environment surrounding the cells.

It was thus found that it is possible to treat a CNS damage or deficit after an insult by increasing the number of stem cells or progenitor derived cells including neurons, astroglial cells and oligodendrocytes.

It was also found that it is possible to treat neural loss suffered after a CNS insult by increasing the number of stem cells or progenitor derived cells including neurons, astroglial cells and oligodendrocytes in a patient by increasing the concentration of GH in the patient to induce proliferation and/or differentiation of stem cells with a concomitant increase in cell genesis.

Finally it was found that it is possible to treat neural loss suffered after a CNS insult by increasing the number of stem cells or progenitor derived cells including neurons and/or astroglial cells and/or oligodendrocytes in a patient by increasing the concentration of GH in the patient to induce proliferation and/or differentiation of stem cells with a concomitant increase in cell genesis in order to facilitate the isolation through surgical removal of small samples of brain tissue containing said cells for further expansion in vitro and concomitant re-transplantation into the patient.

Thus, the present invention relates to the use of a substance that upon administration to a patient will lead to an increased concentration of growth hormone, or of an analogue thereof, for the production of a medicinal product for treatment of an abnormal condition affecting neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells, as well as to a method for treatment of an abnormal condition affecting neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells, wherein a pharmaceutically active amount of a substance that will lead to an increased concentration of growth hormone is administered to a patient.

The substance that will lead to an increased concentration of growth hormone or analogue thereof may e.g. be growth hormone itself, or a functionally equivalent analogue thereof. The term "functionally equivalent analogue thereof" relates to all substances that upon administration to a patient will have essentially the same biologi-

cal and pharmaceutical effect as GH. Such an analogue may e.g. be a synthetic GH mimetic. It is also possible to use a compound that upon administration to a patient will give rise to an elevated active concentration of GH or of a natural occurring GH analogue or its mediators in the CNS of the patient, e.g. by giving rise to an increased release of endogenous GH. For example, positively regulating binding proteins of GH may be used, such as the GH releasing substance growth hormone releasing peptide (GHRP) and analogous thereof.

The medicinal product according to the invention preferably comprises the active substance in a pharmacologically acceptable carrier or diluent such as those known in the art.

The medicinal product or the substance used according to the invention is preferably administered via intravenous periferal infusion or via intramuscular or subcutaneous injection into the patient. It is also possible to administer the medicinal product or the pharmaceutically active substance through a surgically inserted shunt into a cerebral ventricle of the patient.

Preferably, the administered subcutaneous dosage range of the pharmaceutically active substance is about 0.01-1 IE/kg body weight of the patient per week.

The term "patient", as used herein, relates to any human or non-human mammal in need of treatment according to the invention.

The term "treatment" used herein relates to both treatment in order to cure or alleviate a disease or a condition, and to treatment in order to prevent the development of a disease or a condition. The term treatment also refer to the affecting of cell genesis from stem cells or progenitor cells, by inducing the genesis of neurons and/or glial cells after either neuronal, oligodendroglial or glial cell loss in the CNS or PNS (periferal nervous system) or to prevent the normal age related deterioration in the CNS or PNS, the term also relates to

the cultivation of stem or progenitor cells for concomitant transplantation to the CNS or PNS in patients. The treatment may either be performed in an acute or in a chronic way.

As stated above the pharmaceutically active substance used according to the invention is suitable for treatment of abnormal conditions affecting neural stem cells, progenitor cells and/or cells derived from neural stem cells or progenitor cells. It can thus be used to prevent, treat or ameliorate damages, diseases or deficits of central nervous system (CNS). The pharmaceutically active substance used according to the invention is especially suitable for treatment of conditions affecting the oligodendroglia, astroglia, and/or neuronal cells. Such conditions may e.g. be a CNS damage or deficit, neuronal cell loss or memory loss. Such conditions may be caused by a number of different factors or diseases, such as multiple sclerosis, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, and demyelition disorder.

The effect the pharmaceutically active substances used according to the invention is due to their ability to either induce cell genesis, proliferation and/or differentiation of progenitor derived cells in or from the central nervous system.

According to another embodiment of the invention it is possible to use growth hormone or a functionally equivalent analogue thereof in order to propagate progenitor cells or stem cells or other neural cells in a tissue culture or a cell culture. Such cells may thereafter be used for cell transplantation into a patient suffering from neuronal cell loss or a condition due to lack of endogenous cells of this type. The cells used to start the culture may either originate from the patient itself of from human or animal donors.

When cells are to be removed from a patient for in vitro propagation it may be advantageous to first in-

crease the number of progenitor cells in the patient. This facilitating the subsequent isolation of said cells from patients facilitates the subsequent isolation of said cells from patients. The number of progenitor cells are increased by use of the method or medicinal product according to the invention, i.e. by the use of substance that upon administration to a patient will lead to an increased concentration of growth hormone or a functionally equivalent analogue thereof.

Growth hormone, or a functionally equivalent analogue thereof, may be used alone or in junction with other medicaments or growth factors such as epidermal growth factor (EGF) or fibroblast growth factor 2 (FGF2) designed to induce in cell genesis or proliferation in the CNS or PNS. Growth hormone, or a functionally equivalent analogue thereof, alone or in conjunction with other medicaments, peptides, growthfactors, steroids, lipids, glycosylated proteins or peptides, either simultaneous or in sequence, may be used in order to facilitate cell genesis or the generation of specific cell types in vivo or in vitro. It may also be used to induce immature, or multipotent cells to active specific developmental programs as well as specific genes in the aforementioned cells.

By the above mentioned cell genesis is meant the generation of new cells such as neurons oligodendrocytes schwancells and astroglial cells from multipotent cells, progenitor or stem cells within the adult CNS or PNS or in vitro.

Furthermore, the invention also relates to the therapeutic use of substances that decrease the amount of active GH or naturally occurring analogous of GH in the patient and thus decrease the genesis of oligodendrocytes in patients with axonal or spinal cord injury. Examples of such substances are negatively regulating binding proteins, GH-receptor antagonists, drugs or antibodies or compounds or peptides. Axonal regeneration and spinal

cord injury have been shown to be inhibited by certain molecules expressed by oligodendrocytes. Furthermore, drugs or antibodies or compounds or peptides, that increase endogenous peptides, or proteins that decrease the biological activity of endogenous GH can also be used.

The invention will be more fully understood when reading the following example. It should not, however, be considered to limit the scope of the invention.

Brief description of the drawings

In the examples below, reference is made to the appended drawings on which:

Fig. 1 shows the density of BrdU-positive cells after 7 days in the dentate gyrus of hypophysectomized (Hx) rats treated according to the invention with growth hormone (GH), together with cortisol (C), and L-thyroxine (T) compared to hypophysectomized rats treated with only C and T, and to a control group.

Fig. 2 shows that animals treated with cortisone, thyroxine and GH, according to the invention, had significantly more granulae cell neurons than hypophysectomized animals treated with only cortisone and thyroxine four weeks after the last BrdU injection the hypophysectomized animals

Fig. 3 shows that animals with increased number of new born cells according to the invention (○) performed significantly better in the hidden-platform version of the water maze task, used to assess spatial performance, than a control group (●).

Examples

In this example, the density of BrdU-positive cells in the dentate gyrus of hypophysectomized (Hx) rats treated according to the invention with growth hormone (GH), cortisol (C), and L-thyroxine (T) was compared to

the density of BrdU-positive cells in the dentate gyrus of hypophysectomized rats treated with cortisol (C), and L-thyroxine (T), and to the density of the same cells for an untreated unoperated control group.

Fisher rats (Harlan Sprague Dawley) which were intact or hypophysectomized at 50 days of age were maintained under standardized conditions of temperature (24-26°C), humidity (50-60%) and with lights on between 0500 and 1900 h.

The rats had free access to standard laboratory chow and water. Hormonal treatment started 7-10 days after hypophysectomy. All the hypophysectomized rats were given cortisol phosphate (400 µg/kg/day; Solu-Cortef, Upjohn, Puurs, Belgium) and L-thyroxine (10 µg/kg/day; Sigma, USA) diluted in saline as a daily subcutaneous injection (at 0800 h). Recombinant bovine GH (bGH) was diluted in 0.05 M phosphate buffer, pH 8.6, with 1.6% glycerol and 0.02% sodium azide. GH 1 mg/kg/day was given as one daily subcutaneous injection at 24 h intervals. The treatment continued for seven days. Thereafter the rats were sacrificed and the brains taken out and prepared for immunohistochemistry.

Ten hypophysectomized rats were substituted with only cortisole and L-thyroxine. Fifteen hypophysectomized rats were substituted with cortisole, L-thyroxine and GH. Ten rats weighing 120 g were assigned to a control group. During the seven days of the treatment period all animals received a daily intraperitoneal injection (50 mg/kg bodyweight) of bromodeoxyuridine (BrdU; Sigma). The thymidine analog BrdU is incorporated into the genetic material upon mitotic division whereafter it can be detected immunohistochemically in the resulting cells. On the twentieth day all animals were sacrificed by a lethal dose of anesthetics and transcardially perfused with 4% paraformaldehyde. The brains were removed and postfixed in 4% paraformaldehyde for 24h. and thereafter stored in 30% sucrose solution. Coronal freezing microtome sections

(40μm) were stored in cryoprotectant (25% ethylene glycol, 25% glycerin, 0.05 M phosphate buffer) at -20°C until processing for immunohistochemistry or immunofluorescence.

The number of BrdU positive cells in the dentate gyrus of the hippocampus were counted using unbiased counting techniques. For detection of BrdU-labeled nuclei in tissue sections, the following DNA denaturation steps preceded the incubation with mouse anti-BrdU antibody 1:400 (Boeringer Mannheim) : 2 h incubation in 50% formamide/2X SSC (0.3 M NaCl, 0.03 M sodium citrate) at 65°C, 5 min. Rinse in 2 x SSC, 30 min incubation in 2N HCl at 37°C, and 10 min. Rinse in 0.1M boric acid, pH 8,5. All stainings were performed on free floating 40 mm sections. Free-floating sections were treated with 0.6% H_2O_2 in tris buffered saline (TBS) (0.15M NaCl, 0.1M Tris-HCl, pH 7.5) for 30 min to block endogenous peroxidase. Several rinses in TBS were then followed by incubation in TBS/0.25% Triton X-100/3% normal horse serum (TBS-TS) for 30 min and incubation with primary antibody in TBS-TS overnight at 4°C. After rinsing in TBS-TS, the sections were incubated for 3 hr with biotinylated horse anti-mouse IgG, 1:160 secondary antibodies (Vector Laboratories, USA). After TBS rinsing avidin-biotin-peroxidase complex was applied for 1 h followed by peroxidase detection for 5 minutes (0.25 mg/ml diaminobenzidine, 0.01% H_2O_2, 0.04% NiCl).

For the immunofluorescence, sections were treated for DNA denaturation as described above, followed by incubation in TBS-TS for 30 min. Thereafter the sections were incubated with mouse-anti-Calbindin-D28k, 1:2000 (Sigma) for 16 h at 4°C and was detected with a Texas red conjugated donkey anti-mouse IgG. BrdU was detected with a FITC conjugated rabbit anti-BrdU antibody. Fluorescent signals were detected and processed using a confocal scanning laser microscope (Bio-Rad MRC1024, Richmond, CA).

The total number of BrdU positive cells in the granule cell layer, the subgranular layer and the hilus and their corresponding sample volumes were determined in 7-9 coronal sections, 240 mm apart, that contained the dentate gyrus. Cell counting was done according to an optical dissector method to avoid over sampling errors.

The results are shown in figure 1. After 7 days, the number of newborn cells in the dentate gyrus is significantly increased in hypophysectomized animals substituted with GH, cortisone, and thyroxine compared to animals substituted with only cortisone and thyroxine. Furthermore, the rate of proliferation was significantly increased after administration of GH to hypophysectomized animals treated with cortisol and L-thyroxine as quantified after one week of substitution. These results clearly show that GH increase the proliferative rate of progenitor cells in the dentate gyrus in the hippocampus.

Furthermore, the rate of proliferation was significantly increased after administration of GH to hypophysectomized animals treated with cortisol and L-thyroxine as quantified after one week of substitution. This result suggest that GH affect the proliferative rate of progenitor cells in the dentate gyrus of the hippocampus.

Furthermore, the proliferation was increased in normal animals receiving treatment for one week with GH compared with normal controls and compared with the hypophysectomized animals that were substituted for one week and thereafter unsubstituted during the following 3 weeks. The number of BrdU positive cells were estimated one month after treatment with either cortisone and L-thyroxine or cortisone, L-thyroxine and GH. The results are shown in figure 2.

The results suggest that GH either direct or indirect promote proliferation or survival of cells resulting from neural cell progenitor proliferation in the dentate gyrus.

The inventors of the present invention are the first to show that growth hormone can regulate the proliferation and subsequent generation of neurons in the adult brain.

Rats with increased number of newborn cells were tested and compared with rats that had lower number of newborn cells four weeks after BrdU injection during four consecutive days. The rats were tested in a water maze with a video-tracking system. The time to reach the platform (latency) were monitored. The escape platform was hidden 1 cm below the surface of the water at a fixed position. The water was made opaque by adding dry milk powder to the water. The water temperature was kept constant at 22°C throughout the test. Each animal was tested in four trials each day. Each trial lasted 45 s. Animals that failed to find the hidden platform within 45 s were designated as having a 45-s latency and were put on the platform and allowed to stay there for 15 s.

The latency in finding the platform during the water maze test was analyzed with a two-way ANOVA, and repeated postcomparative tests at each monitored time interval were performed using the Scheffe *F*-test. The results are shown in figure 3. There were no significant difference in swim speed. It is evident that animals with increased number of newborn cells in the dentate gyrus, due to treatment according to the invention, performed significantly better in the spatial learning task. These group of animals represent the data denoted with ○ in the figure. The data for the rats with the lower number of newborn cells are denoted with ● in the figure.

CLAIMS

1. Use of a substance that upon administration to a patient will lead to an increased concentration of growth hormone or a functionally equivalent analogue thereof for the production of a medicinal product for treatment of a CNS damage affecting neural stem cells, progenitor cells and/or cells derived from stem cells or progenitor cells.

2. Use according to claim 1, wherein said substance is growth hormone or a functionally equivalent analogue thereof.

3. Use according to claim 1, wherein said substance upon administration will increase the release of endogenous growth hormone.

4. Use according to any one of the claims 1-3, wherein said CNS damage affects the oligodendroglia, astroglia, and/or neuronal cells.

5. Use according to any one of the claims 1-4, wherein said CNS damage affects non-cholinergic neuronal cells, cholinergic neuronal cells, or glial cells.

6. Use according to any one of the claims 1-5, wherein said CNS damage is neural cell loss.

7. Use according to any one of the claims 1-6, wherein said CNS damage is caused by hypoxic injury, ischemic injury, and/or traumatic injury.

8. Use according to any one of the claims 1-7, wherein said medicinal product is formulated for intravenous infusion, intramuscular injection or subcutaneous injection.

9. Use according to any one of the claims 1-8, wherein said medicinal product is formulated so that the active substance will pass into the ventricles of the patient's brain when it is administered to a patient.

10. Use according to any one of the claims 1-9, wherein said medicinal product is formulated so that the

active substance will pass into the cerebrospinal fluid of the patient when it is administered to a patient.

11. Use of a substance that upon administration to a patient will lead to a decreased concentration of growth hormone or a functionally equivalent analogue thereof for the production of a medicinal product for treatment of an abnormal condition affecting the central nervous system, wherein said abnormal condition is the consequence of axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, damage to the spinal cord after disease and/or trauma.

12. Use according to claim 11, wherein said substance is a negatively regulating growth hormone binding protein, a functionally equivalent analogous thereof, an antibody against growth hormone, a biologically active growth hormone receptor inhibitor, and/or an inhibitor of endogenous growth hormone release.

13. A method of propagating progenitor cells, stem cells and/or cells derived from said cells by administration of an effective amount of growth hormone or a functionally equivalent analogue thereof to stem cells, progenitor cells, neurons astroglial cells and/or oligodendrocytes in vitro.

14. A method of inducing lineage determination or inducing or maintaining the genesis of neurons, oligodendrocytes, astroglial cells from progenitor cells or stem cells in, or derived from, the central or periferal nervous system in a patient, wherein a pharmaceutically effective amount of a substance that will lead to an increased concentration of growth hormone or a functionally equivalent analogue thereof is administered to said patient.

15. A method according to claim 14, wherein said substance is growth hormone or a functionally equivalent analogue thereof.

16. A method according to claim 14, wherein said substance is a substance that increases the release of endogenous growth hormone.

17. A method according to claim 14, for treatment of an abnormal condition affecting the nervous system of a patient.

18. A method according to claim 17, wherein said condition affects the oligodendroglia, astroglia, and/or neuronal cells.

19. A method according to claim 17, wherein said condition affects the non-cholinergic neuronal cells, cholinergic neuronal cells, or glial cells.

20. A method according to claim 17, wherein said condition is a CNS damage or deficit.

21. A method according to claim 20, wherein said condition is neural cell loss.

22. A method according to claim 20, wherein said condition is memory loss.

23. A method according to claim 20, wherein said condition is caused by at least one factor selected from the group consisting of multiple sclerosis, hypoxic injury, ischemic injury, traumatic injury, Parkinson's disease, and demyelinating disorder.

24. A method according to claim 14, wherein said substance is administered by intravenous infusion, intramuscular injection or subcutaneous injection.

25. A method according to claim 14, wherein brain cells are removed from the patient after said administration, said brain cells then being propagated in vitro, followed by transplantation of the obtained cells back into the brains of the patient.

26. A method according to claim 25, wherein an effective amount of growth hormone or a functionally equivalent analogue thereof is administered to said brain cells during in vitro propagation.

27. A method of reducing the genesis of oligodendrocytes, neurons, astroglial cells from progenitor cells or

stem cells in, or derived from, the central or periferal nervous system in a patient, wherein a pharmaceutically effective amount of a substance that will lead to a decreased concentration of growth hormone or a functionally equivalent analogue thereof is administered to said patient.

28. A method according to claim 27, wherein said substance is administration to the periferal or central nervous system of said patient.

29. A method according to claim 27, wherein said substance is selected from the group consisting of negatively regulating growth hormone binding proteins, functionally equivalent analogous thereof, antibodies against growth hormone, biologically active growth hormone receptor inhibitors, and inhibitors of endogenous GH release.

30. A method according to claim 27, for treatment of a central nervous system injury.

31. A method according to claim 30, wherein said injury is the consequences of a factor selected from the group consisting of axonal damage caused by concussion, axonal damage caused by head trauma, axonal damage caused by small vessel disease in the CNS, damage to the spinal cord after disease or trauma.

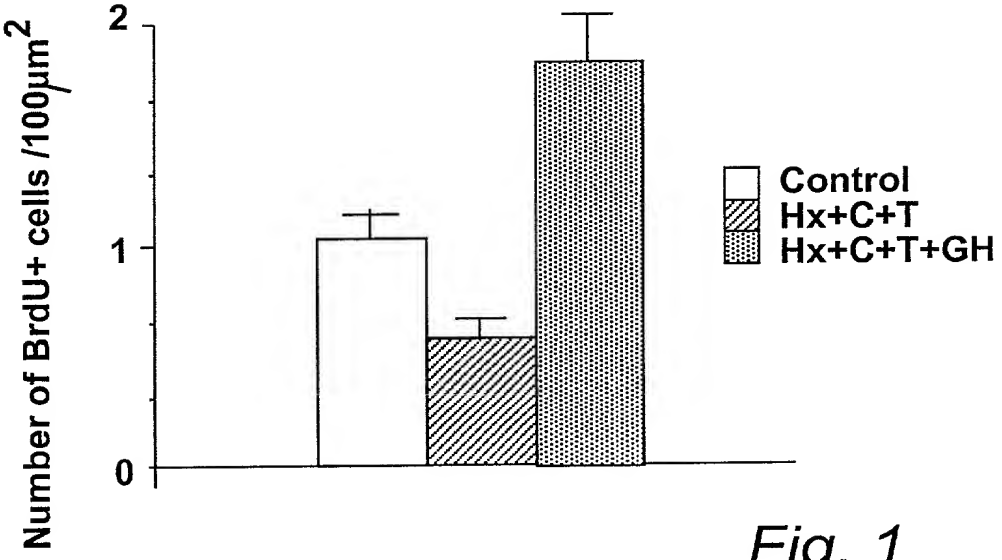
Number of BrdU+ cells /100μm2
2
1
0
Control
Hx+C+T
Hx+C+T+GH

Fig. 1

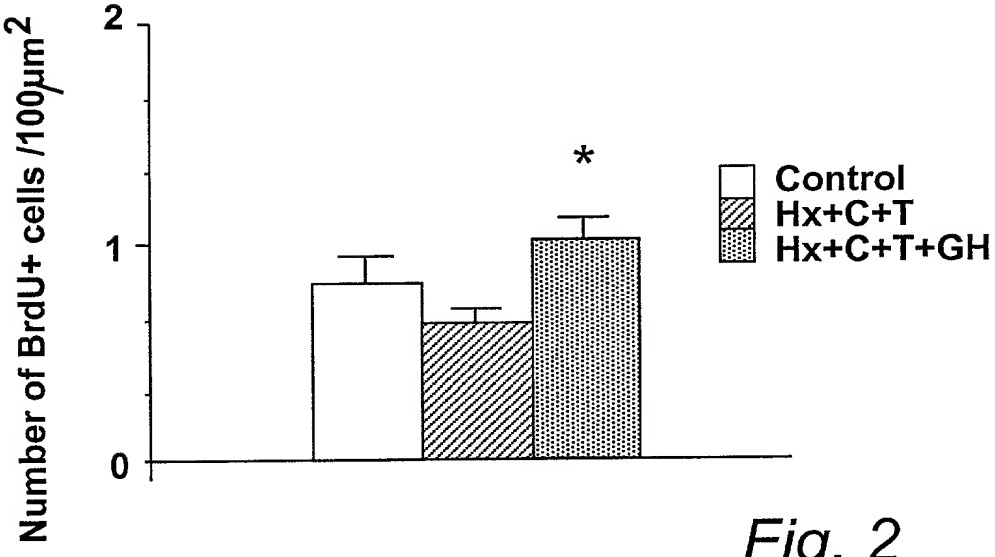
Number of BrdU+ cells /100μm2
2
1
0
*
Control
Hx+C+T
Hx+C+T+GH

Fig. 2

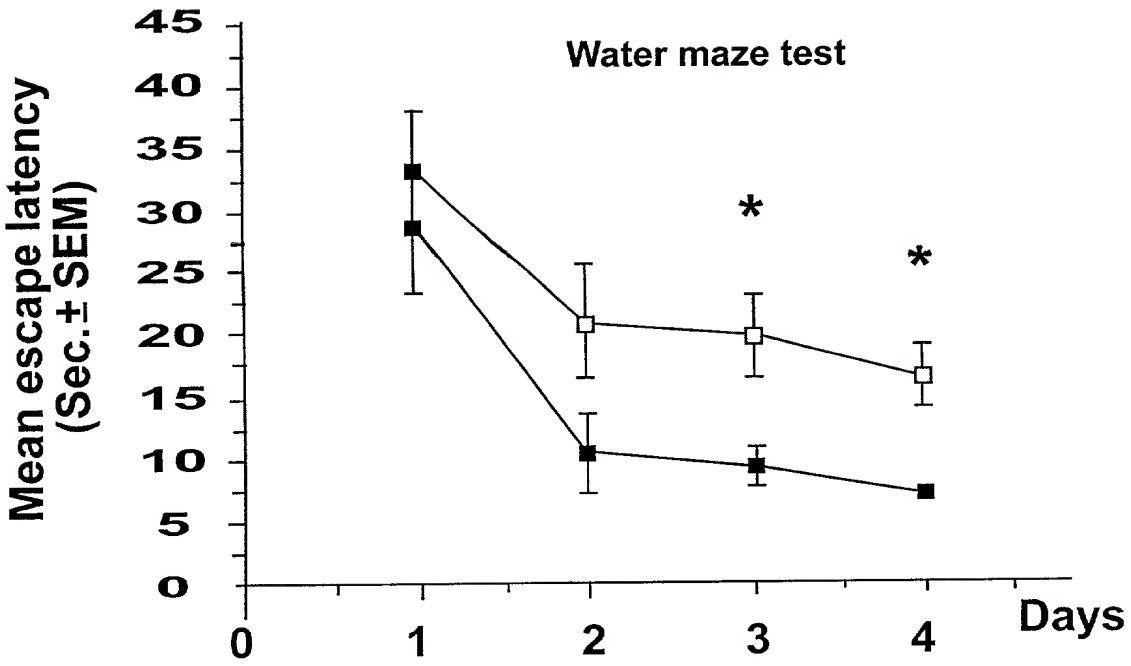
Water maze test
Mean escape latency
(Sec.± SEM)
45
40
35
30
25
20
15
10
5
0
0
1
2
3
4
Days
*
*

Fig. 3

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-782

As a below named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

MEDICINAL PRODUCT AND METHOD FOR TREATMENT OF CONDITIONS AFFECTING NEURAL STEM CELLS OR PROGENITOR CELLS

the specification of which (check only one item below):

☐ is attached hereto.

☐ was filed as United States application
Number _____
on _____
and was amended
on _____ (if applicable).

☒ was filed as PCT international application
Number PCT/SE99/02197
on November 25, 1999
and was amended
on February 12, 2001 (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose to the Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(e) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. §119:

COUNTRY (if PCT, indicate "PCT")	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED UNDER 35 U.S.C. §119
Sweden	9804064-5	25 November 1998	_ Yes X No
			_ Yes _ No
			_ Yes _ No
			_ Yes _ No
			_ Yes _ No

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

(05/01)

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY (CONT'D) (Includes Reference to Provisional and PCT International Applications)	Attorney's Docket No. 003300-782

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose to the Office all information known to me to be material to the patentability as defined in Title 37, Code of Federal Regulations §1.56, which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. APPLICATIONS OR PCT INTERNATIONAL APPLICATIONS DESIGNATING THE U.S. FOR BENEFIT UNDER 35 U.S.C. §120:

U.S. APPLICATIONS		STATUS *(check one)*		
U.S. APPLICATION NUMBER	U.S. FILING DATE	PATENTED	PENDING	ABANDONED

PCT APPLICATIONS DESIGNATING THE U.S.					
PCT APPLICATION NO.	PCT FILING DATE	U.S. APPLICATION NUMBERS ASSIGNED (if any)			

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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